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Effects of Temperature on Spore Germination in Some Fern Species from Semideciduous Mesophytic Forest

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ABSTRACT.—Spore germination in eight fern species from semideciduous mesophytic forest in the State of São Paulo, Brazil, was studied under laboratory conditions at four different temperatures. For most species, the shortest average germination times were observed at the three higher temperatures. The germinability was similar at all temperatures tested for *Polypodium hirsutissimum, P. latipes,* and *Pteris denticulata*. Higher germinability was observed at average temperatures of 18.4, 21.7, and 25.2 °C for spores of *Microgramma lindbergii, M. squamulosa,* and *Polypodium polypodioides,* and was observed at 21.7, 25.2, and 29.4 °C for spores of *Adiantopsis radiata* and *Polypodium pleopeltifolium*. In nature, germination probably occurs mainly in November and December, when spores are abundant in the environment, water supplies are ample, and temperature conditions are most suitable.

Few detailed studies have investigated the effects of temperature on fern spore germination (Miller, 1968; Dyer, 1979; Raghavan, 1980, 1989). Sensitivity to temperature is known to vary from one species to another. It is generally related to temperature requirements for subsequent normal gametophyte development and to the natural distribution of the species (Hevly, 1963; Raghavan, 1980, 1989; Warne and Lloyd, 1980; Pérez-García and Riba, 1982). The area of spore dispersal may be greater than the area in which sporophytes are recorded (Page, 1979), and temperature appears to be among the limiting factors that determine establishment of the species in the environment.

The effect of temperature on spore germination in certain fern species is complex because of interactions with light (Raghavan, 1980, 1989). High temperatures following irradiation with a saturating dose of red light inhibit the spore germination process. Raghavan (1980, 1989) stated that high temperatures inactivate phytochrome molecules. According to Towill (1978), degree of hydration and changes in membrane properties play some part in the temperature sensitivity of the spores. Darkness, or darkness in combination with low temperature, used as pretreatments, stimulated spore germination in *Schizaea pusilla* Pursh during subsequent light treatment (Guiragossian and Konning, 1986).

Some ferns and their ecophysiological relationships have been studied in a semideciduous mesophytic forest of southeastern Brazil in the Barreiro Rico forest. *Microgramma lindbergii, M. squamulosa, Polypodium hirsutissimum, P. latipes, P. pleopeltifolium,* and *P. polypodioides* occur in places exposed to greater thermic and humidity oscillations than the habitats of *Adiantopsis radiata* and *Pteris denticulata* (Ranal, 1995a). The species in the first group have more morphological and physiological characteristics associated with desic-

cation tolerance than do the others (Ranal, 1991a, b; Ranal, 1993). The peak in growth of these species in this forest occurs between October and April, whereas from May to September their metabolism is reduced as a result of lower temperatures and water availability (Ranal, 1995a).

The major cause of mortality of these species is desiccation (Ranal, 1995b). New leaves and spores in these species are produced during the wet season. Spore dispersal of *Polypodium hirsutissimum, P. pleopeltifolium,* and *P. polypodioides* occurs during the dry season (April to August), whereas for the other species dispersal occurs during the wet season (January to March) and the beginning of the dry season (April).

These data, associated with information on spore germination, are important to an understanding of the mechanisms whereby the eight species can establish themselves in the Barreiro Rico forest. In this sense, this paper reports the effects of four different temperatures on spore germination in eight fern species from a semideciduous mesophytic forest in the State of São Paulo, Brazil.

Materials and Methods

Eight species of ferns from a semideciduous mesophytic forest on the Barreiro Rico Farm, municipality of Anhembi, State of São Paulo, Brazil (22°41′S, 48°07′W, 560 m altitude) were selected for the present study: *Microgramma lindbergii* (Kuhn) Sota, *M. squamulosa* (Kaulf.) Sota, *Polypodium hirsutissimum* Raddi, *P. pleopeltifolium* Raddi, and *P. polypodioides* (L.) Watt. are epiphytes; *Adiantopsis radiata* (L.) Fée, *Polypodium latipes* Langsd. et Fisch., and *Pteris denticulata* Sw. are terrestrial species. Voucher specimens are deposited in the Herbarium Rioclarense (HRCB) of the Universidade Estadual Paulista (UNESP) Campus at Rio Claro. They were determined by Dr. Paulo G. Windisch according to the classification system of Tryon and Tryon (1982).

The region is characterized by Koeppen Cwa type climate with a dry winter from April to September and a wet summer from October to March (Ranal, 1995a). The average temperature of the coldest month during the period 1970–1985 was 17.5°C (July) and of the warmest month (February), 25.2°C (Ranal, 1995a).

The fertile leaves of five species were left for 24 hours in wax paper bags at room temperature (ca. 25.0°C), and the released spores were collected in glass vials closed with cork stoppers. They were stored at 4.0°C until preparation of the cultures. For *Polypodium hirsutissimum, P. pleopeltifolium,* and *P. polypodioides,* which have sori covered with scales, the spores were collected over a period of 48 hours.

Samples of the stored spores were washed in sterile distilled water and separated from sporangia and other impurities by centrifugation at 975 rpm. The densities of the spore inoculum were obtained by counting the number of spores in 0.07 ml of suspension spread over 1 cm^2 area of a microscope slide using five replicates (Table 1). The spore suspensions were kept for 40 hours at 24.0°C for imbibition in darkness, and the culture medium was then inoculated using Pasteur pipettes.

TABLE 1. Date of collection, spore age, and average concentration of spore suspensions for eight fern species.

Experiment	Species	Collection	Spore age (days)	Average concentration (spores/ml)
1	*Microgramma lindbergii*	15 Jan 1982	25	3028
	M. squamulosa	15 Jan 1982	25	3143
	Polypodium polypodioides	04 Nov 1981	97	2614
	Pteris denticulata	15 Jan 1982	25	3214
2	*Adiantopsis radiata*	15 Jan 1982	206	3086
	Polypodium hirsutissimum	28 Aug 1982	8	2428
	P. latipes	16 Apr 1982	213	2614
	P. pleopeltifolium	07 May 1981	481	2428

The cultures were prepared in 50×20 mm Petri dishes, with 5 ml solid (1% bactoagar, DIFCO) culture medium of Mohr (1956), as modified by Dyer (1979). As suggested by Dyer (1979), one percent Mycostatin (E.R. Squibb; 10,000 units/ml) was added to the culture medium as a fungicide. No contamination was observed in the cultures. After inoculation with 0.5 ml of spore suspension, the cultures were kept in germination chambers under four different temperatures (Table 2). Light was supplied by two white fluorescent lamps of 20 W (daylight) and four white incandescent lamps of 5 W installed 22 cm above the culture dishes, producing an average irradiance of 874 $\mu W \cdot cm^{-2}$, 12 hours per day. During the remaining 12 hours, cultures received diffuse indirect artificial light from the laboratory. The irradiance was measured using an Optronic radiometer, model 730A.

Germination data were obtained at intervals of 24 hours for approximately 90 days. Statistical analysis was done with data obtained after 12–13 days of culture, when maximum germination had been observed. The criterion for germination was the emergence of the rhizoid or chlorocyte (the first chlorophyllous cell of the young gametophyte). Four areas of 1 cm^2 per treatment were analysed.

The percentages of germination were submitted to arc sine transformation (arc sine $\sqrt{x/100}$, where x is the percentage) before analysis of variance and Tukey tests were carried out (Schefler, 1969). The average germination time was determined according to Labouriau (1983), using the total number of spores from each treatment ($t = \Sigma n_i t_i / \Sigma n_i$). The analysis of variance and Tukey test for this parameter were carried out with the Statistical Package for Social Science (SPSS V.H.), using n_i (number of spores germinated per day) as the weight for t_i (time of observation).

RESULTS AND DISCUSSION

Germination began between the second and sixth days after the inoculation of the spores onto the culture medium (Table 3). Germination of the spores of the eight species studied occurred within the limits (1–14 days) observed by

TABLE 2. Temperatures (mean ± standard error) recorded during fern spore germination.

Experiment	Temperature (°C)			
	Chamber 1	Chamber 2	Chamber 3	Chamber 4
1	18.5 ± 0.1	22.2 ± 0.2	25.2 ± 0.2	29.8 ± 0.1
2	18.3 ± 0.1	21.2 ± 0.1	25.3 ± 0.1	29.0 ± 0.1

Sussman in 1965 for most leptosporangiate ferns (Howland and Edwards, 1979). Four species showed the shortest time for 50% germination at 25.2 and/or 29.4°C (Table 3). Two species, *Adiantopsis radiata* and *Microgramma squamulosa,* did not reach this rate of germination at any temperature studied. In the majority of the species, the shortest average germination times were observed at the three higher temperatures (Table 4).

The eight species can be grouped according to the germination curves (Figs. 1–8). In *Adiantopsis radiata, Polypodium hirsutissimum, P. latipes, P. pleopeltifolium,* and *Pteris denticulata,* spore germination is inhibited (low germinability) or delayed (slow germination) at 18.4°C. In *Microgramma squamulosa* and *Polypodium polypodioides,* inhibition and delay occur at 29.4°C. In *Microgramma lindbergii,* delay in germination occurs at 18.4°C and inhibition was observed at 29.4°C.

At the end of 12 or 13 days, greatest germinability was observed at 18.4, 21.7, and 25.2°C for spores of *Microgramma lindbergii, M. squamulosa,* and *Polypodium polypodioides,* and it was observed at 21.7, 25.2, and 29.4°C for spores of *Adiantopsis radiata* and *Polypodium pleopeltifolium.* No significant effect of temperature in the range used was observed for *Polypodium hirsutissimum, P. latipes,* and *Pteris denticulata* (Table 5).

Two temperatures, 21.7 and 25.2°C, were the most favorable for this first phase of gametophyte development. Similar results to this range of temperatures have been obtained for spores of *Ceratopteris thalictroides* (L.) Brongn. and *C. pteridoides* (Hooker) Hieron. (Warne and Lloyd, 1980), for some species of Cyatheaceae and Lophosoriaceae (Pérez-García and Riba, 1982), *Cyathea delgadii* Sternb. (Marcondes-Ferreira and Felippe, 1984), *Trichipteris corcovadensis* (Raddi) Copel. (Esteves et al., 1985), and *Polypodium latipes* (Esteves and Felippe, 1988).

Polypodium hirsutissimum, P. latipes, and *Pteris denticulata* are generalists—G in relation to germination temperature. In *Microgramma lindbergii, M. squamulosa,* and *Polypodium polypodioides,* spore germination is inhibited by high temperatures—IHT (29.4°C and probably above), whereas in *Adiantopsis radiata* and *Polypodium pleopeltifolium,* spore germination is inhibited by low temperatures—ILT (18.4°C and probably lower).

The distribution of the populations of adult sporophytes in the different microhabitats of the Barreiro Rico forest does not in all cases correspond to this laboratory information. *Microgramma lindbergii, M. squamulosa, Polypodium hirsutissimum, P. latipes, P. pleopeltifolium,* and *P. polypodioides* are established in microhabitats with a broader range of temperature and air hu-

TABLE 3. First germination and time for 50% of germination (days after inoculation) for fern spores at different temperatures. Temperatures are the average temperatures of experiments 1 and 2. F indicates the first germination observed and the dash means that 50% of germination was not reached.

Species	Temperature (°C)							
	18.4		21.7		25.2		29.4	
	F	50%	F	50%	F	50%	F	50%
Microgramma lindbergii	5	—	4	8	4	12	4	—
M. squamulosa	5	—	4	—	4	—	4	—
Polypodium hirsutissimum	3	9	2	7	2	6	2	6
P. pleopeltifolium	6	13	2	8	2	6	2	6
P. polypodioides	5	—	4	11	4	—	4	—
Adiantopsis radiata	4	—	3	—	3	—	3	—
Polypodium latipes	5	8	4	8	4	7	4	8
Pteris denticulata	5	12	5	8	4	8	4	7

midity than the microhabitats of *Adiantopsis radiata* and *Pteris denticulata* (Ranal, 1995a). The range of temperature registered for 11 fern microhabitats in the Barreiro Rico forest, from March 1985 to May 1986, was 4.5–38.5°C (Ranal, 1995a). The extreme temperatures occurred in gaps or in the upper parts of the trees during part of the day; the lower (4.5–15.0°C) from April to September and the higher (above 35.0°C) in October and November. The range of air humidity was 30–100%. Values below 50% were registered in open places in the forest, during the period from May to July (winter), and in October and November (summer), from 1:30 to 3:30 p.m. (Ranal, 1995a).

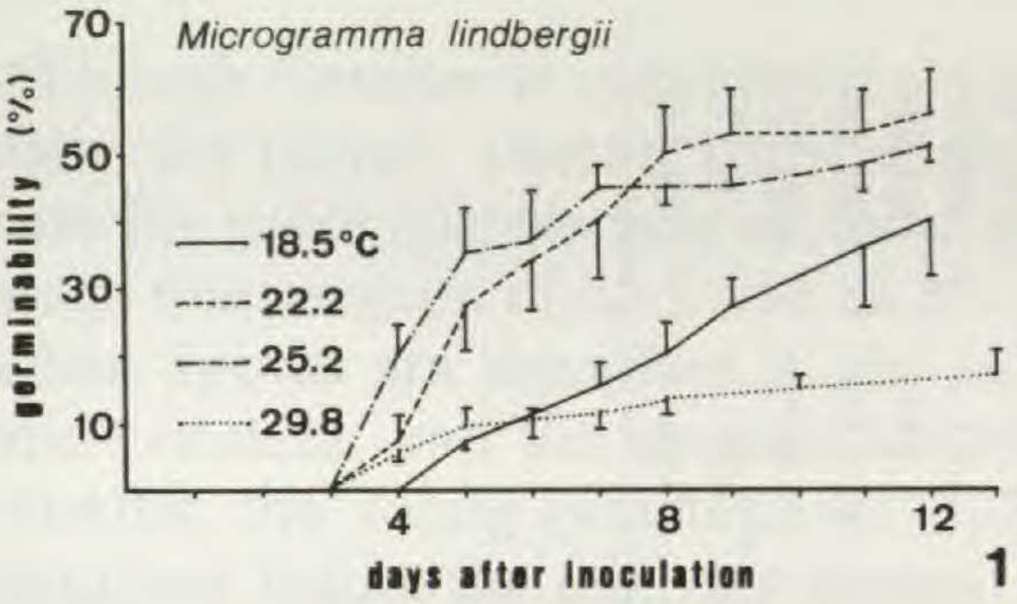
Spores of *Microgramma lindbergii, M. squamulosa,* and *Polypodium polypodioides* do not germinate well at 29.4°C under laboratory conditions. The small number of gametophytes formed at this temperature remained at the filamentous stage (Ranal, 1983). Under natural conditions, these spores may well germinate in protected places in the forest; only after the appearance of the sporophyte can the creeping stem reach open places where the adult sporophytes are established. Sporophytes of these species, especially the two last cited, are resistant to hydric stress (Ranal, 1991b; Ranal, 1995a) with adaptation to open places in the forest.

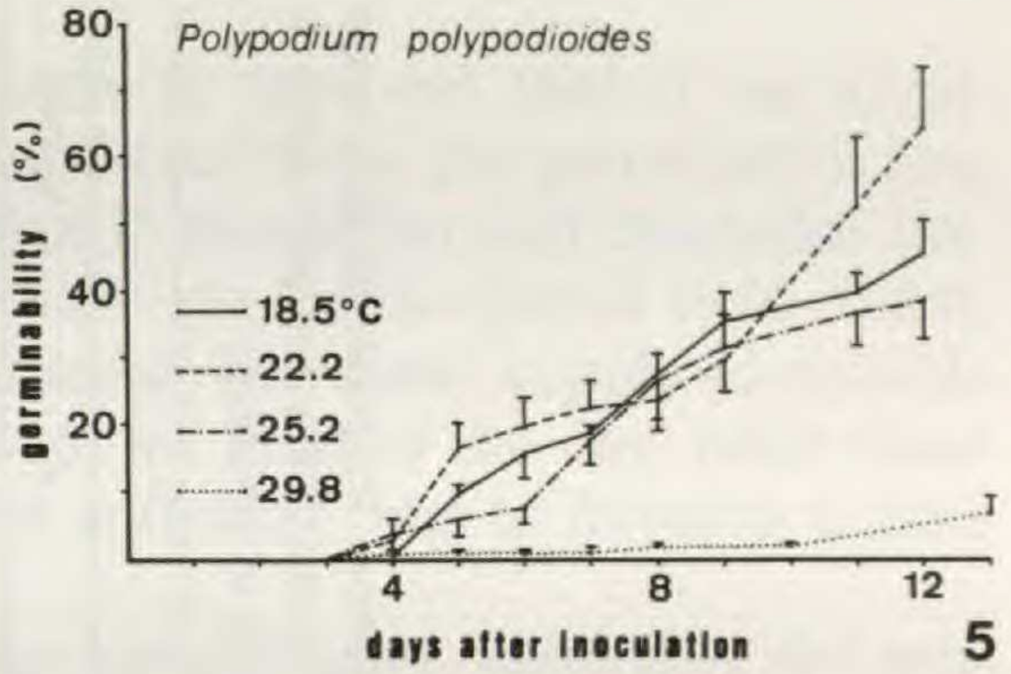
In natural conditions, *Microgramma lindbergii*—IHT and *M. squamulosa*—IHT (epiphytic species), *Adiantopsis radiata*—ILT, *Polypodium latipes*—G, and *Pteris denticulata*—G (terrestrial species) release their spores from January to April. *Polypodium hirsutissimum*—G, *P. pleopeltifolium*—ILT, and *P. polypodioides*—IHT (epiphytic species) release their spores from April to August (Ranal, 1995b). For all the species included in this paper, there was no apparent relationship between life forms, time of spore dispersion, and sensitivity to germination temperature.

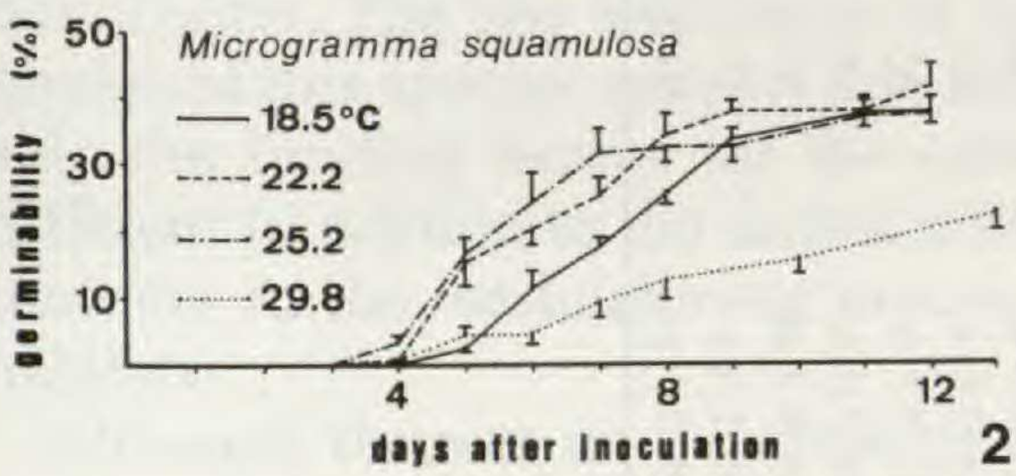
During the warm wet season (October to March), temperatures restrictive to germination of the species studied were registered in March (18.0°C), between midnight and 10:30 a.m., and November (14.5–18.0°C), between 3 and 6 a.m. (Ranal, 1995a). For the other months, the minimum temperature was 19.5°C.

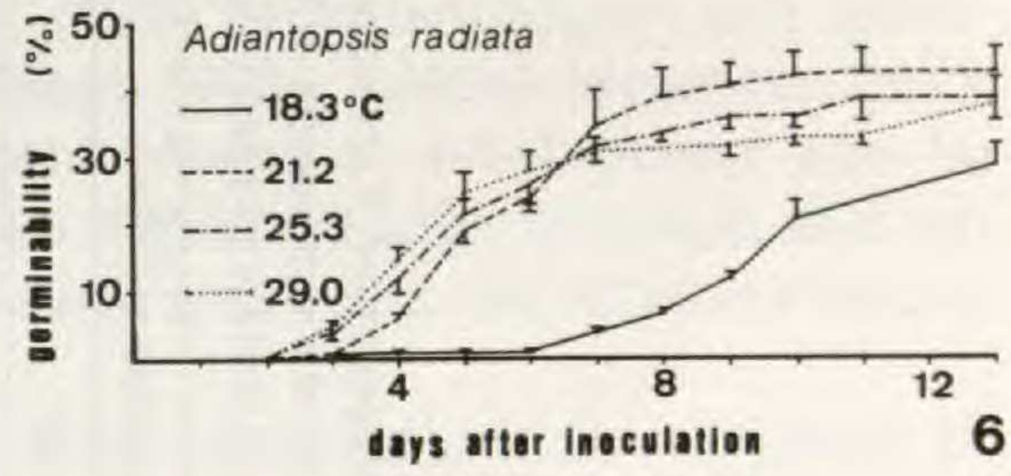
TABLE 4. Average germination times (mean ± standard error; days) of fern spores at different temperatures. Temperatures are average temperatures of the experiments 1 and 2. Means followed by the same letter in each line are not significantly different based on the Tukey test ($\alpha = 0.05$). F indicates the values of Snedecor's distribution (** significance at 1% of probability) and d.f. the degrees of freedom.

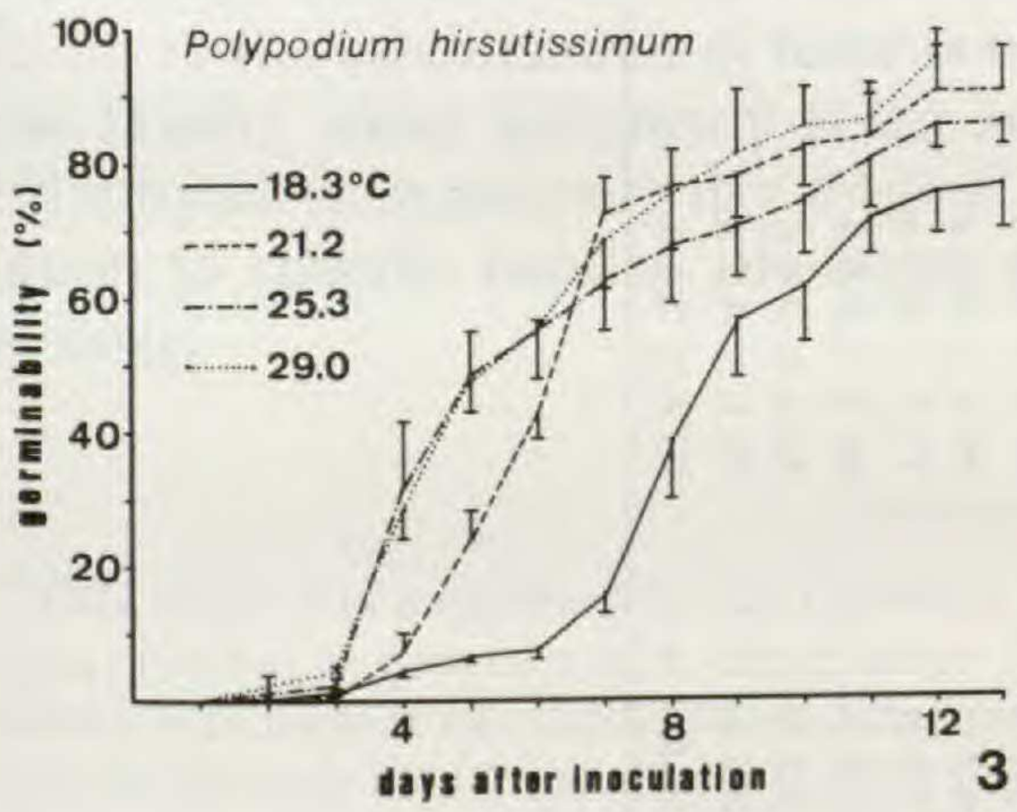
Species	Temperature (°C)					
	18.4	21.7	25.2	29.4	d.f.	F
Microgramma lindbergii	8.4 ± 0.2 b	6.2 ± 0.2 a	5.8 ± 0.2 a	6.6 ± 0.3 a	3;579	40.74**
M. squamulosa	7.7 ± 0.1 c	7.1 ± 0.1 b	6.4 ± 0.1 a	8.8 ± 0.2 d	3;857	42.57**
Polypodium hirsutissimum	8.5 ± 0.1 d	6.8 ± 0.1 c	5.8 ± 0.2 a	6.4 ± 0.1 b	3;1382	78.37**
P. pleopeltifolium	9.1 ± 0.2 d	7.0 ± 0.1 c	6.3 ± 0.1 b	5.6 ± 0.1 a	3;1314	80.56**
P. polypodioides	8.1 ± 0.2 a	8.9 ± 0.2 b	7.8 ± 0.2 a	11.5 ± 0.6 c	3;499	15.0**
Adiantopsis radiata	10.1 ± 0.2 b	6.2 ± 0.1 a	5.9 ± 0.1 a	6.2 ± 0.2 a	3;1212	166.21**
Polypodium latipes	8.2 ± 0.02 d	7.4 ± 0.04 b	6.6 ± 0.03 a	7.8 ± 0.05 c	3;7309	545.05**
Pteris denticulata	9.3 ± 0.1 d	7.5 ± 0.1 c	6.4 ± 0.1 a	7.0 ± 0.1 b	3;1157	130.43**

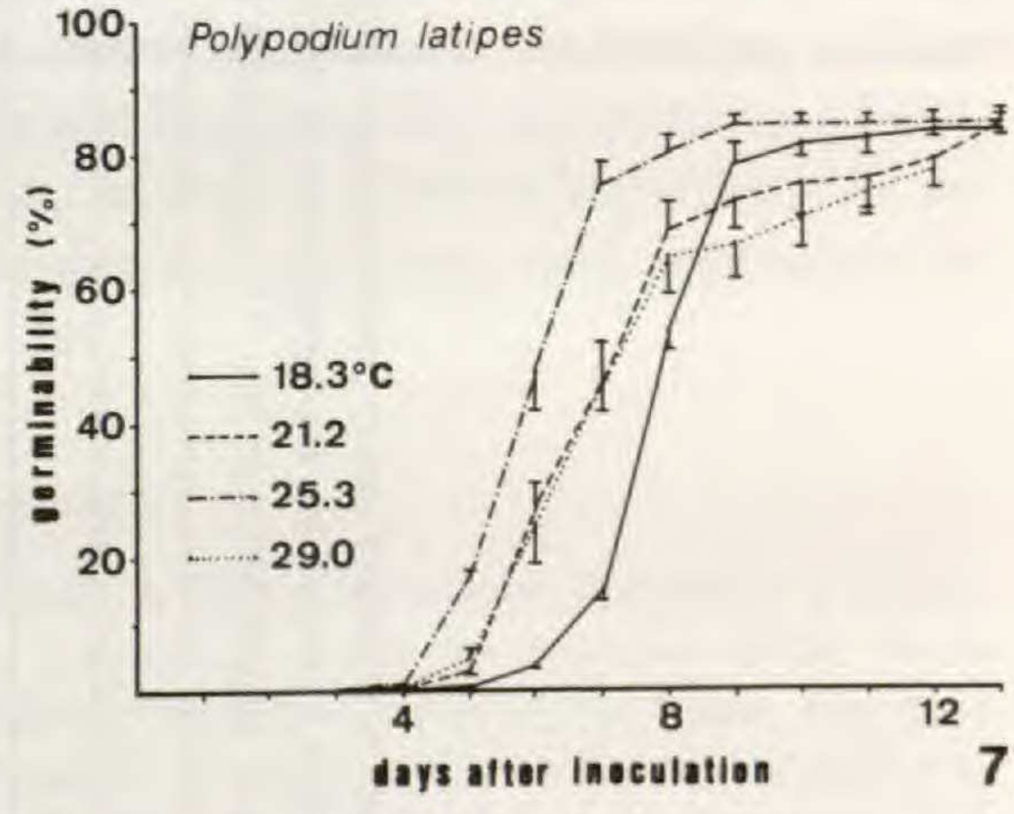


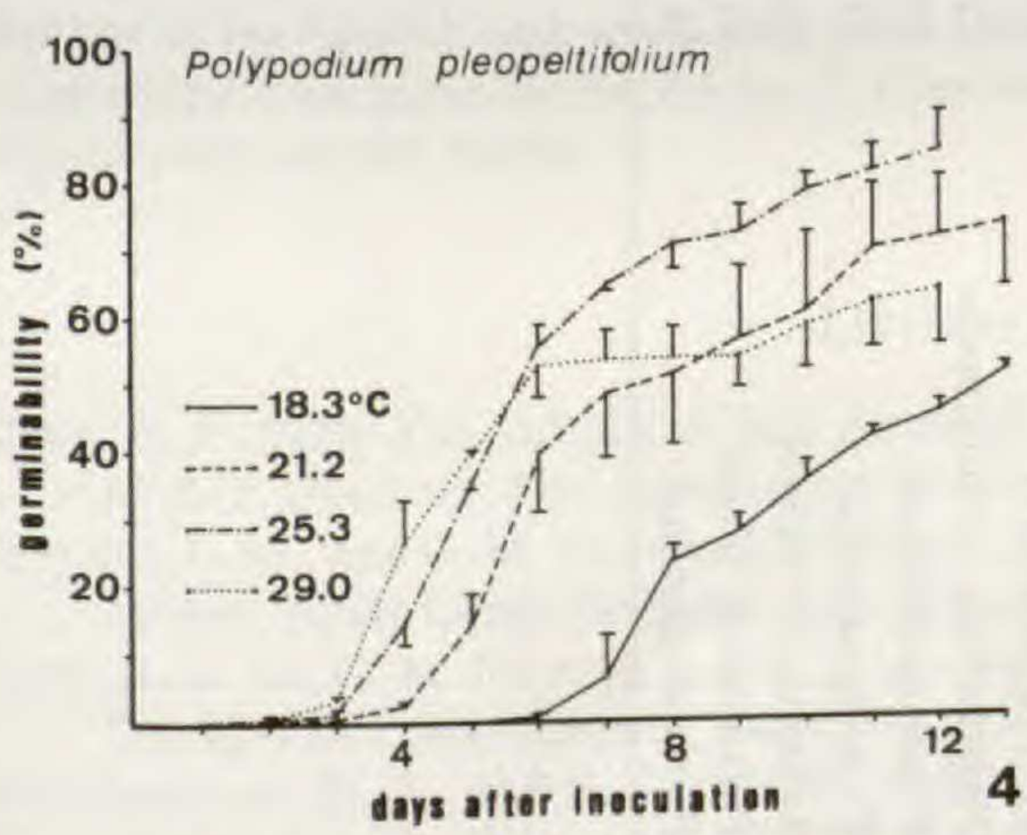


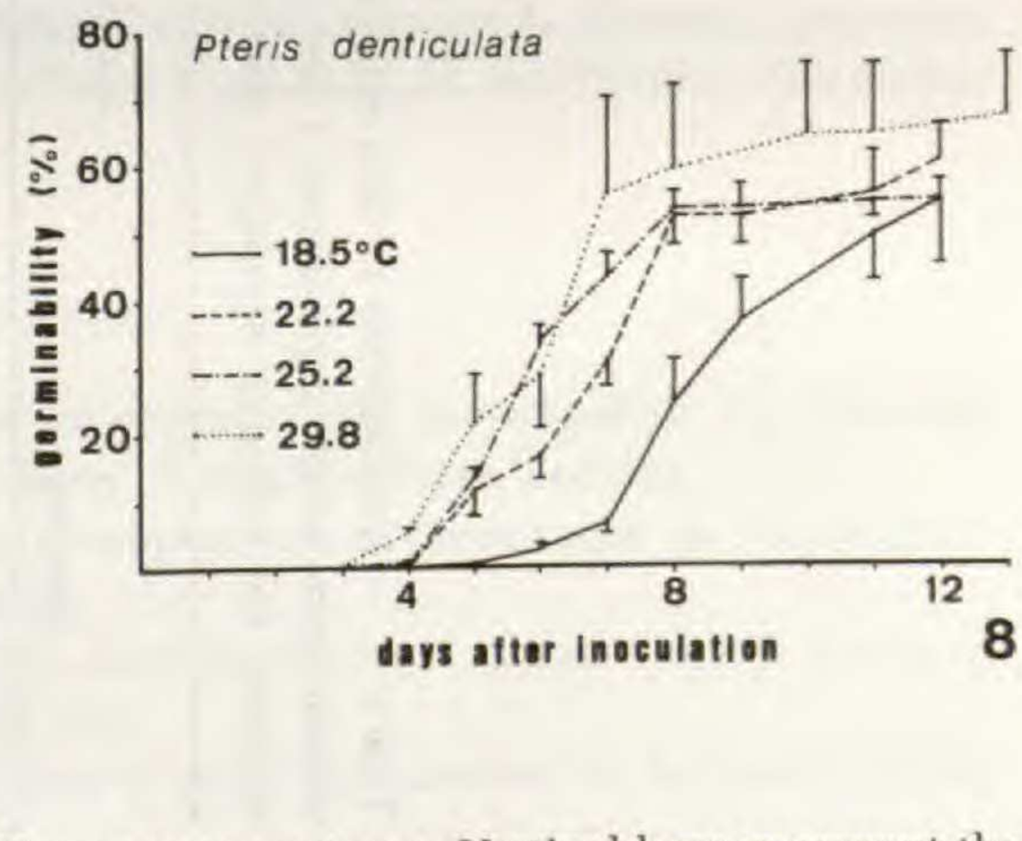












FIGS. 1–8. Germinability of fern spores at four different temperatures. Vertical bars represent the standard errors of the means.

TABLE 5. Germinability of fern spores (mean ± standard error; percentage) at different temperatures, 12–13 days after inoculation. Temperatures are average temperatures of the experiments 1 and 2. Means followed by the same letter in each line are not significantly different based on the Tukey test ($\alpha = 0.05$). F indicates the values of Snedecor's distribution (* significative at 5%, ** significative at 1% of probability) and C.V. the coefficient of variation.

Species	Temperature (°C)					
	18.4	21.7	25.2	29.4	F	C.V. (%)
Microgramma lindbergii	40.6 ± 8.2 a	56.5 ± 6.4 a	52.0 ± 2.6 a	17.7 ± 3.1 b	10.13**	17.10
M. squamulosa	37.9 ± 2.3 a	41.5 ± 3.2 a	37.6 ± 1.5 a	22.8 ± 2.8 b	11.08**	8.73
Polypodium hirsutissimum	75.8 ± 6.3 a	90.7 ± 6.6 a	85.6 ± 3.6 a	95.7 ± 4.0 a	2.81	14.47
P. pleopeltifolium	45.3 ± 1.6 b	71.7 ± 8.7 ab	84.1 ± 5.7 a	63.3 ± 7.7 ab	5.14*	17.92
P. polypodioides	45.8 ± 5.5 a	64.3 ± 9.6 a	38.7 ± 5.4 a	6.7 ± 2.7 b	17.33**	21.52
Adiantopsis radiata	28.4 ± 3.2 b	42.6 ± 3.9 a	38.7 ± 3.3 ab	37.8 ± 2.2 ab	3.64*	10.31
Polypodium latipes	83.9 ± 0.9 a	79.1 ± 4.7 a	84.7 ± 1.6 a	77.9 ± 2.7 a	1.26	6.37
Pteris denticulata	54.7 ± 10.0 a	60.9 ± 5.1 a	54.5 ± 2.7 a	67.2 ± 9.4 a	0.66	19.34

Although October is considered a wet month, in 1984 and 1985 it was atypically dry (Ranal, 1995a). Thus, under natural conditions the germination process for these species may be most effective in November and December (average temperature of 23.3 and 24.0°C, respectively, for the period 1970–1985), when spores are abundant in the environment, the water supply is suitable and constant, and the temperature adequate for most of the day. After these months, the young gametophyte will have sufficient time to develop a plate structure before the next dry season.

The results of this study suggest that for some species, especially the generalists, other factors are probably more limiting in their establishment than habitat temperatures, at least in the range of temperatures studied. For example, the humidity of the environment may be the limiting factor for *Pteris denticulata*. The low endurance of hydric stress by gametophytes and sporophytes of this species support this idea (Ranal, 1991a, 1995a). Moreover, probably the limiting factors for the subsequent phases of development may be different in relation to the factors which are important to germination and can interfere in the establishment process of each species in the different microhabitats.

Although there is no quantitative data about gametophyte development in seasonally dry tropical areas, Barreiro Rico included, the results obtained in this study indicate that in these environments the most important limiting factor to the establisment of ferns is water, not temperature. According to Kornás (1985), water deficiency is the key factor limiting the occurrence of pteridophytes in seasonally dry tropical areas, acting on their adaptations in relation to specific habitat, life-forms, phenological patterns, and reproductive biology.

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